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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [5]:
C12N 15/48, A61K 37/02, C12N 5/10, G01N 33/50, C12P 21/00, C07K 15/04, C12P 21/08

A2

(11) International Publication Number: **WO 94/20621**

(43) International Publication Date: 15 September 1994 (15.09.94)

(21) International Application Number: PCT/GB94/00281

(22) International Filing Date: 11 February 1994 (11.02.94)

(30) Priority Data:
9304239.8 1 March 1993 (01.03.93) GB

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(81) Designated States: AU, CA, CN, DE, FI, GB, JP, KR, NO, NZ, RU, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: PROTEINACEOUS PARTICLES CONTAINING RETROVIRAL ELEMENT

(57) Abstract

A DNA construct comprising (a) a single promoter; (b) a *gag*-derived sequence from a complex retrovirus; (c) a *rev*-like element; (d) an RRE-like element; and (e) donor and acceptor elements; wherein (i) elements (a) to (e) are arranged in such a fashion that in the construct that the promoter is capable of driving expression of both the *gag*-derived sequence and, by virtue of the donor and acceptor elements, the *rev*-like element; (ii) the construct does not contain a functional env gene.

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PROTEINACEOUS PARTICLES CONTAINING RETROVIRAL ELEMENTS.

The present invention relates to proteinaceous particles and their expression in mammalian cells. Proteinaceous particles have previously been proposed in the preparation of vaccines. Correct presentation of an antigen to an animal's immune system is a key requirement for an effective sub-unit vaccine or immunogen. The assembly of the antigenic determinants into a high molecular weight complex or particle appears to be significant in enhancing an effective immune response. The particles can be used for targeting antigens or as delivery systems for therapeutic agents.

An ideal immunogen is a polymer of multiple antigen determinants assembled into a high molecular weight, particulate complex. A substantial disadvantage of most antigens produced by recombinant DNA techniques for vaccines is that they are usually produced as simple monomeric proteins. This is not the ideal configuration of an immunising antigen as it does not readily permit the cross-linking of the components of the immune system that is required for maximum stimulation of humoral and cellular immunity. For these reasons it would be advantageous to develop polyvalent, particulate carrier systems for immunising antigens.

Prior to the present invention a number of self-assembling antigen presentation systems have been developed. These include systems based on fusion of antigens to the Hepatitis core antigen (Beesly *et al*, 1990 *Bio/Technology* 8, 644-649), Hepatitis B surface antigen (Valenzuela *et al*. 1985, *Bio/Technology* 3, 323), Tobacco mosaic virus coat protein (Haynes et al, 1986, *Bio/Technology* 4, 637), poliovirus (Burke *et al*, 1988 *Nature* **332**, 81-82) and yeast retrotransposon Ty1 p1 (WO-A-8803563 and WO-A-8803562). All of these systems form multimeric fusion protein complexes.

Similar self-assembling fusion systems have been developed for the *gag* genes of HIV-1, where retroviral Gag proteins have been fused to a heterologous gene (WO-A-8803563 and WO-A-8803562). GB 9208218.9 discloses a Gag:V3 particulate

fusion protein, while Weldon et al, in *J. Virol.* **64** (9) 4169-4179 (1990), disclose fusion particle formation using the Rous sarcoma virus *gag* gene, Pr76^{gag}, fused to iso-1-cytochrome c from *Saccharomyces cerevisiae*. Although expression of such Gag particles has been carried out in a number of different replication systems, including yeast, insect and mammalian cells, expression in mammalian cells could be optimised.

The efficient expression of Gag particles in a mammalian system has a number of advantages, including, but not limited to, effective post-translational modifications which more closely resemble the processing observed in the native retroviral system. In particular, glycosylation patterns in insect and yeast cells are different from mammalian systems and conform less to the native system. Glycosylation contributes to the folding and stability of the protein, and may be important in cellular sorting, budding and targeting. The correct presentation of carbohydrate moieties may also enhance immune recognition of the protein.

The expression of Gag particles from mammalian cells rather than insect cells also has several advantages for product registration. Insect cell-derived biological products have not yet been licensed for clinical use and the co-purification and contamination of Gag particles with baculovirus can be avoided. It would therefore be highly desirable to improve the expression of Gag particles in mammalian cells.

The regulatory systems controlling the expression of HIV are highly complex and not fully understood (Haseltine & Wong-Staal, 1988, *Scientific American*, **259**, 34-42; Haseltine, 1988, *Journal of Acquired Immunodeficiency Syndrome*, **1**, 217-240). The genomic organization of HIV is similar to that of other complex retroviruses, having seven genes in addition to *gag*, *pol* and *env* (Rosen *et al*, 1990, *AIDS*, **4**, 499-509), Mechanisms exist for both upregulation and inhibition of expression of HIV proteins (Maldarelli *et al*, 1991, *J. Virol.*, **65**, 5732-43).

The complicated RNA splicing strategy used by HIV (Feinberg *et al*,., Cell **46** 807-817 (1986) is also shared by other complex retroviruses (Cullen et al., J. Virol.

(1991) 65 (3) 1053-1056). Feinberg *et al.* also describes the Rev function which controls the switch between expression of control proteins and structural proteins. The early stages of HIV replication are marked by the exclusive expression of fully spliced mRNA species that encode the viral regulatory proteins Tat, Rev and Nef. Two of these regulatory genes, *tat* and *rev* are produced from overlapping reading frames by multiply spliced messenger RNAs. Tat increases the steady state levels of all HIV-1 messenger RNAs and interacts with the *cis*-acting responsive sequence known as TAR.

The *rev* gene is involved in a shift in expression from regulatory proteins to structural proteins. Raised levels of the *rev* gene product lead to a marked shift in viral gene expression, whereby the predominant expression product becomes the incompletely spliced transcripts that encode the viral structural proteins, including Gag and Env. Rev function is mediated through a unique *cis*-acting element found in the *env* region of HIV-1, known as the Rev-responsive element or RRE (Rosen *et al*, 1990, *AIDS* **4** (6), 499-509). Efficient virion/Gag particle production may require the presence of the *cis*-acting RRE nucleic acid sequence and co-expression of the *trans*-acting Rev protein (Cochrane *et al*, 1991, *J. Virol.*, **65**, 5305-5313; D'Agostino, 1992, *Mol. & Cell. Biol.*, **12**, 1375-86). Mergener *et al.*, (1992) *Virology*, **186**, 25-39 showed that RRE is important for Gag particle formation in mammalian cells. Rev is also important in viral infectivity (Adachi *et al.*, Arch Virol **117** 45-58 (1991)); Rev- mutants are non-infectious. The importance of splicing is unclear (Rosen *et al*, 1990).

Expression may be further enhanced by use of the Tat-TAR interaction, which has been the subject of much debate in the art (Rosen *et al*, 1990, *AIDS* **4** (6), 499-509; Cullen J. Virol. **65**(3) 1053-1056 (1991)). Tat has been used to control the expression of heterologous promoters (Han *et al.*, NAR **19**(25) 7225-7229 (1991)). While the use of the Tat/TAR interaction might improve the efficiency of Gag expression, it is possible that the use of a strong promoter such as the CMV promoter might be sufficient. Alternatively, the HIV-LTR sequences, which contain the TAR site, might be used as a promoter sequence in conjunction with the Tat

element.

The art also suggests that in addition to Gag p17, p24 and p7, p6 and protease may be important in particle formation and extracellular budding (Gottlinger *et al.*, 1991, *Proc. Natl. Acad. Sci. USA*, **88**, 3195-3199.)

Studies with infectious carrier systems bearing HIV have been undertaken, usually based on a live vaccinia expression system (Karacostas *et al*, 1989, *Proc. Natl. Acad. Sci. USA*, **86** 8964-8967; Shioda & Shibuta, 1990, *Virology* **175** 139-148; Haffar et al, J. Virol. 64 (6) 2653-2659 and Vzoror et al, 1991, *AIDS Research and Human Retroviruses* **7** (1) 29-36). Other live viral systems have been used, notably recombinant fowlpox virus (FPV) (Jenkins *et al*, 1991, *AIDS Research and Human Retroviruses* **7** (12) 991-998) using SIV genes for the production of recombinant defective virus-like particles. In this instance this method involves the co-infection of a cell with two separate viruses - one FPV vector containing the SIV *env* gene and the other FPV vector carrying the *gag-pol* genes of SIV. Co-expression resulted in the generation of particles bearing SIV envelope glycoprotein. While this process, known as phenotypic mixing, can generate multivalent particulate carriers bearing a heterogeneous membrane protein, it has a number of disadvantages. All such preparations contain infectious virus (except in the case of FPV), which renders them undesirable for clinical use, in particular for immunocompromised patients. Inactivation of the active virus may destroy the biological activity of the protein and the antigenicity of the epitopes. The production process for the manufacture of such particles is highly expensive and difficult to control, with a high risk of mutagenesis in the culture medium.

There are also many examples of expression of non-infectious clones or sub-viral constructs of HIV in mammalian cells, which lead to the production of structurally mature particles (Lever *et al.*, 1989 *J. Virol.* **63** (9) 4085-4087; Haynes *et al*, 1991, *AIDS Research and Human Retroviruses* **7** (1) 17-27). However, these strategies have either involved the expression of full-length HIV clones or the separate expression of portions of the HIV genome in tandem with several different promoter

systems, with one promoter being used for each separate gene region. The highest level of Gag particle expression is obtained when Rev is expressed from spliced transcripts (Haynes *et al*, 1991).

Whilst such expression strategies have been successful in producing immunogenic virus-like particles, there are a number of disadvantages for both product registration and manufacturing purposes. Firstly, a variety of other active components of the HIV replication cycle are often expressed simultaneously, including reverse transcriptase (Smith *et al*, 1990 *J. Virol*, **64** (6) 2743-2750), integrase and RNA which are unsuitable for therapeutic use. Hoshikawa *et al*, (*J. Gen.Virol.* **72** 2509-2517 (1991)) note that the protease region of the *pol* gene is required for morphological maturation of HIV particles although complete proteolytic cleavage of the Gag protein may prevent bud formation. Such particles, potentially carrying replicating DNA, would encounter difficulties in obtaining regulatory approval, both due to uncertainty about safety and the necessity for assaying for the presence of all viral proteins. Secondly, where separate gene sequences are expressed independently in a single cell, multiple promoter systems are required for expression. One obvious way to express only the desired open reading frames (ORFs) has been to clone the cDNA copies of HIV transcripts which express each of the desired ORFs into mammalian cell expression vectors and to combine these clones in a single stable cell line (for example Vernon *et al.* (J. Gen. Virol. **72** 1243-1251 1991) used adenovirus vectors encoding Gag p55 expressed from one promoter and Rev expressed from a second promoter). However, this requires the co-expression of HIV Gag, Tat and Rev. Including a selectable marker, four heterologous transcripts from four promoters might have to be coordinately expressed in the cell line. This is technically demanding and the efficiency of expression of these virus-like particles in mammalian cells is poor (Moosmayer *et al*, 1991, *Virology*, **183** 215-224). This system is therefore unsuitable for large-scale manufacture. It would also be difficult to ensure consistency between batches using multiple promoter systems.

WO-A-9107425 (Oncogen) discloses, inter alia, the production of recombinant

proteins carrying Gag and Env, together with Tat, Rev and the N-terminal portion of Pol. In this plasmid, expression of all proteins is driven by a HIV-derived promoter.

Although this plasmid expresses a significantly reduced number of proteins compared to the native virus, while retaining Rev and RRE control of Gag production, there are a number of ways in which expression could be improved. Firstly, an improved yield of Gag particles would be desirable. Secondly, it would be desirable to reduce the amount of HIV-derived DNA, so as to improve safety aspects and decrease analytical time and costs. However, it is by no means certain that removal of potentially important DNA sequences would not adversely affect Gag production since removal of those sequences might have important conformational consequences, or disrupt splice patterns.

Page et al., discloses an HIV vector which has a 1.2 kb deletion in the *env* gene, the deleted sequence being substituted by a 1.1kb insert comprising a *gpt* gene driven by an SV40 promoter. The result of the substitution in the *env* gene between the components of the *rev* and *tat* elements is that the distance between the coding regions remains substantially unaltered. Further, the use of an additional viral promoter to drive the heterologous gene represents another layer of complexity in the construction of the vector and another possible concern for the regulatory authorities.

There are, therefore, several problems associated with preparing proteinaceous retroviral particles in high yield from mammalian cells. It has not been possible to produce mature Gag particles which are non-infectious and do not simultaneously express portions of the HIV *pol* gene. The production of Gag particles using non-infectious clones or sub-viral clones requires multiple promoter systems if undesirable proteins are to remain unexpressed from open reading frames (ORFs). Several of the expression strategies described in the art were designed to produce recombinant particles which were immunologically identical to the virus, but which were non-infectious and could form the basis for a vaccine preparation. Since these particles contained all the proteins associated with the natural virus (e.g.

reverse transcriptase, integrase, the envelope proteins), there is always a possibility of reversion to wild-type, infectious virus. Sero-conversion profiles generated by vaccination with this type of preparation and by actual infection could be easily confused and elaborate tests would be required to distinguish the two.

Apart from the difficulty in attempting to express the desired ORFs referred to above, another strategy to inhibit expression of the undesirable ORFs would be through single or multiple mutations to alter the ATG translational initiation codons and/or the addition of translational stop codons in all of the undesired ORFs. This approach is feasible (eg 28 point mutations were described in Schwartz *et al.*, J. Virol 66(12) 7176-7182 (1992)) but technically demanding and time-consuming due to the number of subclones for mutagenesis, the number of mutations required and the re-construction of the virus from the subclones. The viral DNA has to be sequenced to ensure the construct is as designed. Reversion is always a possibility, and needs to be monitored by assays for all the components of the HIV genome. Particle formation might also be particularly affected by such an approach.

It would therefore be highly desirable to facilitate high-level production of Gag particles from mammalian cells in the absence of replication competent retroviruses by using a single promoter and near-natural RNA splicing patterns to coordinately express the minimum number of HIV genes for immunogen preparation and, in some embodiments, cellular targetting. The present invention relates to a solution to this problem.

It has been found that Gag particles can be generated where the undesirable ORFs are deleted, while maintaining the splicing pattern of the virus, so that all of the desired ORFs (*gag, tat* and *rev*) are expressed from a single promoter. The desirable segments of the viral genome can be cloned into a multiple cloning site to generate a subviral construct about one-third the size of the virus, making cloning more manageable and the construct easier to sequence. There will also be a reduction in the time and cost of product analysis.

This approach also has a number of product registration advantages by minimising the number of proteins pesent in the vaccine, and may conform more closely to guidelines on quality and safety. Such constructs will be devoid of reverse transcriptase, integrase and envelope proteins and do not encounter the difficulties referred to above.

According to the first aspect of this invention, there is provided a DNA construct comprising:

(a) a *single promoter*;
(b) a *gag*-derived sequence from a complex retrovirus,
(c) a *rev*-like element;
(d) an RRE-like element; and
(e) donor and acceptor elements;

wherein

(i) elements (a) to (e) are arranged in such a fashion that in the construct the promoter is capable of driving expression of both the *gag*-derived sequence and, by virtue of the donor and acceptor elements, the *rev*-like element;

(ii) the construct does not contain a functional env gene

The promoter used could be, for example, derived from the LTR region of a retrovirus such as RSV or HIV. Other suitable promoters for use in mammalian cells include non-inducible promoters (such as the CMV promoter) and inducible promoters (such as the metallothionine promoter).

The retrovirus from which the particle-forming *gag* sequence is derived may be any which possesses functions analogous to the *rev*-like element, namely complex retroviruses (Cullen, 1991, J. Virol. 65 1053-1056). For example *gag* sequences

may be of lentiviral origin such as HIV-1 (also known as HTLV-III or LAV), HIV-2, HTLV-I, HTLV-II, SIV, BIV and FIV, or of spumaretroviral origin such as simian foaming virus, bovine syncitial virus, or feline syncitial virus. More preferred are the Gag sequences from the retroviruses Human Immunodeficiency Virus (HIV) (Gheysen *et al.*, 1989, *Cell* **59**, 103-112), Simian Immunodeficiency Virus (SIV) (Delchambre *et al.*, 1989 *EMBO J.* **8**, 2653-2660) and Bovine Immunodeficiency-like Virus (BIV) (Rasmussen *et al*, 1990, *Virology* **178** 435-451). It is most preferred that the Gag sequence be derived from HIV-1. Stability of particles formed from the fusion proteins so produced may be enhanced by removing some or all of the basic C-terminus of the Gag precursor protein (p55 in HIV-1). At least the first 437 amino acids of the Gag protein will preferably be present in the *preferred truncated form*.

Natural Gag proteins, at least of HIV-1, are post-translationally modified. Such modification usually includes myristylation in HIV-1 Gag. Naturally post-translationally modified Gag proteins and non-post-translationally modified Gag proteins are included within the scope of the invention.

Gag-derived sequences useful in the present invention may code for natural Gag proteins. However, they do not have to: *gag*-derived sequences useful in the invention will generally be substantially homologous to the natural *gag* sequence. For example, the Gag sequence may be supplemented by one or more additional amino acids which need not contribute to the self-assembling ability (although they may do); the additional amino acid(s) may for example contribute to antigenicity. Alternatively or in addition, the sequence of the Gag protein may be modified for other purposes by amino acid substitution, deletion and/or addition. In some embodiments of the invention the nucleic acid binding ability of the protein may be modified. Such a modification may have the effect of reducing or even eliminating the protein's nucleic acid binding activity, for example to help ensure that little or no viral nucleic acid is incorporated in the particle: equally, however, the nucleic acid binding characteristics may simply be modified, rather than reduced, for example so that the particle may be used as a delivery system for nucleic acid with which the

protein is not normally associated. Such nucleic acid includes antisense RNA, RNA with digesting or cleaving activity, such as ribozymes, and DNA and mRNA coding for a biologically active peptide which can be released on site in a target cell; the present invention can therefore be used for gene therapy.

A reference to a "protein" in this specification includes a reference to a primary expression product and, where appropriate, a reference to a post-translationally modified (for example glycosylated) protein.

The expression "substantially homologous", when describing the relationship of an amino acid sequence to a natural protein, means that the amino acid sequence can be identical to the natural protein or can be an effective but truncated or otherwise modified form of the natural protein or can share at least 50%, 60%, 70%, 80%, 90%, 95% or 99%, in increasing order of preference, of the residues of the natural protein or its modified form. Alternatively or in addition, a nucleic acid sequence encoding the amino acid sequence may hybridise, for example under stringent conditions, to a nucleic acid sequence encoding the natural protein or its truncated form, or would do so but for the degeneracy of the genetic code. Stringent hybridisation conditions are known in the art and are exemplified by approximately 0.9 molar salt concentration at approximately 35° to 65°C.

Although the *gag*-derived sequence may comprise a portion of the retroviral polymerase gene, it is preferred that, if it does, only the protease-encoding moiety be present.

The *rev*- like element is also derived from a complex retrovirus (or is substantially homologous thereto); comprising, for example, *rev* when the retrovirus is HIV-1 and *rex* when the retrovirus is HTLV-1. Although HTLV-1 *rex* can activate expression of unspliced HIV-1 mRNA in an HIV-1 RRE dependent manner (Hanly *et al.*, 1989, Genes Dev. 3 1534-44), it is preferred that this element is derived from the same type of retrovirus as the *gag* element.

The RRE-like element is also derived from a complex retrovirus (or is substantially homologous thereto); for example, RRE when the retrovirus is HIV-1 and Rex-RE when the retrovirus is HTLV-1, and is preferably matched to the *rev*- like element.

The selected RNA splicing donor and acceptor sites are positioned in the natural linear sequences but in a spatially compacted configuration to allow natural Rev/RRE- controlled RNA splicing donor/acceptor pairing. The natural Rev/RRE-controlled switch between full length and multiply spliced transcripts is therefore maintained to mimic the normal control of HIV-1 gene expression in this construct. Although the position of RRE changes in different complex retroviruses, the same splice pattern is maintained. The donor and/or acceptor sites will generally be derived rom one or more complex retroviruses (or will be substantially homologous thereto).

A construct which does not contain any functional *env* has a sufficient number of deletions in the *env* gene to stop the protein from being expressed. In particular, it may be desirable to remove CD4 binding activity. This may result in substantially no *env* gene, although enough may remain to take advantage of conveniently placed restriction sites in order to facilitate preparation of the DNA construct. A minimal amount of *env* compatible with convenient manipulation of *rev* and RRE is preferred. The natural *env* gene is 2567 bp in length and deletions of greater than 50% of this sequence are particularly preferred. However, at least the 370bp encoding the RRE region and the 650bp encoding Rev will remain. Such a minimal amount of native *env* would not comprise additional DNA, either non-coding or containing a heterologous gene. The absence of *env* leads to a cleaner construct which may help to satisfy the requirements of the regulatory authorities.

Certain other elements derived from (or substantially homologous to) complex retroviruses may also be present. These include *nef*, *tat*, TAR, *vpu* and *vpr*. The substantial deletion of *env* would bring the *tat* and *rev* sequences into a closer spatial relationship, and it was not certain that their efficacy would be retained. However, it has now been shown that while inclusion of the Tat-TAR interaction

sequences is not necessary for expression of Gag particles, it may be preferred in some cases. While the use of the Tat/TAR interaction might improve the efficiency of Gag expression, it is possible that the use of a strong promoter such as the CMV promoter might be sufficient. Alternatively, the HIV-LTR sequences, which contain the TAR site might be used as a promoter sequence in conjunction with the *tat* element.

It has been shown that immune responses to the Gag proteins of retroviruses have a significant role in the onset and progression of retrovirus induced disease. Therefore, a Gag protein produced by means of this invention may also act as an important immunogenic component of an antigen presentation system, the production of which is one of the uses to which the invention may be put.

The *gag*-derived sequence may optionally be fused in an open reading frame with a further sequence whose expression product is antigenic. This antigenic sequence may be found at the 3' or 5' end of the p6, p7, p24 or p17 region of the *gag* gene, or inserted within the Gag particle-forming sequence, so that the two sequences are expressed as a single fusion protein. In the case of insertions, the insertion site will preferably be an epitope that is surface exposed when the Gag protein forms particles.

The antigenic sequence may correspond to a sequence derived from or associated with an aetiological agent or a tumour. The aetiological agent may be a microorganism such as a virus, bacterium, fungus or parasite. The virus may be: a retrovirus, such as HIV-1, HIV-2, HTLV-I, HTLV-II, HTLV-III, SIV, BIV, LAV, ELAV, CIAV, murine leukaemia virus, Moloney murine leukaemia virus, and feline leukaemia virus; an orthomyxovirus, such as influenza A or B; a paramyxovirus, such as parainfluenza virus, mumps, measles, RSV and Sendai virus; a papovavirus, such as HPV; an arenavirus, such as LCMV of humans or mice; a hepadnavirus, such as Hepatitis B virus; a herpes virus, such as HSV, VZV, CMV, or EBV. The tumour-associated or derived antigen may for example be a proteinaceous human tumour antigen, such as a melanoma-associated antigen, or

an epithelial-tumour associated antigen such as from breast or colon carcinoma.

The antigenic sequence may be also derived from a bacterium, such as of the genus *Neisseria*, *Campylobacter*, *Bordetella*, *Listeria*, *Mycobacteria* or *Leishmania*, or a parasite, such as from the genus *Trypanosoma*, *Schizosoma*, *Plasmodium*, especially *P. falciparum*, or from a fungus, such as from the genus *Candida*, *Aspergillus*, *Cryptococcus*, *Histoplasma* or *Blastomyces*.

Preferred antigenic sequences correspond to epitopes from a retrovirus, a paramyxovirus, an arenavirus or a hepadna virus, or a from human tumour cell. Examples include epitopes from:

1) HIV (particularly HIV-1) gp120,
2) VZV gpI, gpII and gpIII
3) Influenza virus nucleoprotein and haemagglutinin,
4) LCMV nucleoprotein,
5) HPV L1, L2 E4, E5, E6 and E7 proteins,
6) p97 melanoma associated antigen,
7) GA 733-2 epithelial tumour-associated antigen,
8) MUC-1 epithelial tumour-associated antigen,
9) Melanoma MZ2-E antigens,
10) Malaria CSP or RESA antigens,
11) Mycobacterium p6

Particularly preferred antigenic sequences are derived from the third variable domain of a lentivirus. This region of lentiviruses, known as the V3 loop or GPGR loop is found between amino acids 300 and 330 of the envelope glycoprotein gp120 of HIV-1 and in analogous positions of other lentiviruses. The V3 loop is defined by two flanking cysteine residues linked by a disulphide bond and, for HIV-1 at least, is the major neutralising epitope of the virus (Putney *et al* 1986 *Science* 234, 1392; Rusche *et al* 1988 *Proc. Natl. Acad. Sci.* 85, 3198; Palker *et al* 1988 *Proc. Natl. Acad. Sci.* 85 1932; and Goudsmit *et al.*, 1988 *AIDS* 2 157). The

antigenic portion of choice may constitute the whole of the V3 loop wh n derived from different strains. However, multiple copies of a conserved sequence of the V3 loop may be useful in conferring immunity against more than one isolate of a virus (such as HIV-1).

According to a second aspect of the invention, there is provided a process for the preparation of a construct as described above, the process comprising ligating, in one or a plurality of steps, two or more DNA segments between them comprising in any combination:

(a) a single promoter;
(b) a *gag*-derived sequence from a complex retrovirus,
(c) a *rev*-like element;
(d) an RRE-like element
(e) donor and acceptor elements;

the segments being ligated in such a fashion that:

(i) in the construct the promoter is capable of driving expression of both the *gag*-derived sequence and, by virtue of the donor and acceptor elements, the *rev*-like element;

(ii) the construct does not contain a functional *env* gene

It is preferred that the *rev*-like element and the RRE-like element are first ligated together before being joined to the promoter and *gag*-derived sequence.

According to the third aspect of the invention there is provided a DNA construct constisting substantially only of:

(a) a *rev*-like element;
(b) an RRE-like element; and

(c) donor and acceptor elements;

This construct is useful as an intermediate.

Further according to the present invention is provided a vector including nucleic acid as described above. The vector may be derived from a plasmid, for example pWGH (disclosed in WO-A-9109118) where the nucleic acid sequence of interest is linked to a strong promoter and enhancer sequence from cytomegalovirus, a polyadenylation sequence from SV40 and a nucleic acid sequence coding for a selectable marker expressed from an SV40 promoter and having an additional SV40 polyadenylation signal at the 3' end of the selectable marker sequence.

Alternatively the vector may be derived from a live non-oncogenic virus such as recombinant human adenovirus type 5 (Ad5). Live adenovirus vaccines may be administered orally, a route that may be critical in generating immunity to viruses that are mucosally transmitted. The stability, ease of propagation and ease of administration of adenoviruses may make them highly suitable for widespread vaccination programs. Prevec *et al.* (Journal of AIDS, 4 568-576, 1991) describe adenovirus vectors encoding Gag p55 or p24. The vectors did not use the Rev-RRE interaction and, although they generated anti-p24 antibodies in mice and macaques, were inefficient at Gag expression. Vernon *et al.* (J. Gen. Virol. 72 1243-1251 1991) used similar adenovirus constructs, where proteins encoding the Gag precursor p55 and the protease were expressed from one promoter and Rev was expressed from a second promoter. The use of nucleic acid as described above would make the expression more efficient.

The invention also includes mammalian host cells transformed with such vectors, for example COS cells, Chinese Hamster Ovary (CHO) cells, mouse myeloma cell lines such as P3X63-Ag8.653, HeLa cells, BHK cells, VERO cells, melanoma cells such as the Bowes cell line, mouse L cells, human hepatoma cell lines such as HepG2, mouse fibroblasts and mouse NIH 3T3 cells.

As indicated above, DNA constructs in accordance with the first aspect of the invention are useful in the production of *gag*-derived proteins. According to a fourth aspect of the invention, there is provided a process for the preparation of proteins encoded at least in part by a gag-derived gene from a complex retrovirus (or sequence substantially homologous thereto), the process comprising expressing a DNA construct in accordance with the first aspect.

The DNA constructs of the first aspect, either with or without DNA encoding an additional antigenic sequence, may also be used to transform mammalian cells which co-express targeting proteins. Such targeting proteins are expressed as membrane bound proteins and are picked up when the particle buds. A suitable targeting mechanism is described in WO-A-9218621. Preferred membrane-bound proteins confer additional biological properties on the particles. These properties include: conferring an immunogenic ability (or improved or altered immunogenic ability) on the membrane protein; a site- or cell-specific targeting ability; fusogenic properties; binding or packaging activity; cytotoxicity; pharmaceutical activity; and/or nucleic acid binding activity.

If the membrane-bound protein is included because of its immunological properties, then it may be, or include sequences from, a protein from any pathogenic organism, including viruses, fungi, bacteria and protozoa. Preferred membrane bound proteins include those of (or homologus with) viruses, eg the influenza virus haemaggluttinin protein and the *env*-derived proteins of retroviruses, including HIV-1.

In other preferred embodiments, the membrane bound protein will confer upon the particle the property of binding to specified cells or molecules. The cells may be of the immune system and may include antigen-presenting cells (APCs). In this configuration it is expected that an enhanced immune response, both humoral and cellular, will be generated by a given dose of antigen, because a greater proportion of the administered material will be taken up by the cells of interest. When included for this reason, preferred proteins include CD4, CD8, molecules involved in

leukocyte-endothelium and/or leukocyte-leukocyte interactions such as ICAM-1, ICAM-2, ICAM-1ß, VCAM and the adjuvanting/targeting molecule BB1/B7 (the ligand of CD28) as well as antibody molecules, or other specific binding molecules, such as active fragments of antibody molecules, which have specificities for cells of the immune system. Other suitable proteins may be of viral origin such as gp160 of HIV-1, which binds to the T-cell ligand, CD4. Other cell types may also be targetted via the membrane-bound protein. For example, influenza haemagglutinin will target the particles to cells bearing sialic acid on their surface. Ligands to tumour-specific antigens will target the particles to the appropriate tumour.

The targeting proteins may be either naturally expressed, or they may be expressed from another autosomal plasmid vector. They may be stably co-transfected into cells with the *gag* expression vector, or expressed from genes engineered onto *gag* expression vector variants. In this case the targeting protein gene is expressed from another splice acceptor-donor combination within the *gag* expresion construct. The targeted protein would then be expressed as if it were a HIV gene within the deleted virus construct.

Proteins expressed in accordance with the fourth aspect may spontaneously form particles. Such particles may contain a heterologous, or, preferably, homologous population of proteins. Each protein may have any of the configurations described above.

Particles prepared by the method of the invention have many uses. For example, in addition to the use of Gag fusions to carry extra epitopes on the particles, fusions of Gag to active proteins may be envisaged. For example, fusions of genes encoding toxins such as ricin to the *gag* gene may, following packaging with an appropriate targetting protein, allow the particles to deliver toxin to specific cells. Other active proteins might also be used, for example restriction enzymes, drug activating enzymes or Rev BL (a dominant mutant which inhibits viral reproduction (Benko et al., New Biol. 1990 **2** (12) 1111-22; Solomin et al, J. Virol. 1990 **64** (12) 6010-7)).

The *gag* expression cassette has been designed to abrogate the packaging of RNA derived from the expression construct by the deletion of the 19bp packaging site. This allows the possibility of filling the particle with heterologous RNA by co-expression of the *gag* expression cassette and another heterologous expression system into which a DNA encoding the 19bp RNA packaging site has been cloned. This enables the particle to be used as a delivery system for nucleic acid with which the protein is not normally associated. Such nucleic acid includes antisense RNA, RNA with digesting or cleaving activity, such as ribozymes and mRNA coding for a biologically active peptide which can be released on site in a target cell; the present invention can therefore be used for gene therapy. Such heterologous RNA packaging could also help the manufacturing analysis.

Because of the augmented immunogenic nature of the particles produced in accordance with the invention, it is likely that it will be easier to produce antibodies than with conventional antigens and that those antibodies will have specific characteristics. The invention thus further provides antibodies raised against particulate antigens produced in accordance with the invention. The antibodies may be polyclonal (obtained for example by injecting antigens into a rabbit) or monoclonal, produced by hybridoma cells. It is also likely that *in vitro* immunisation can be achieved more readily than with other forms of antigen; this may facilitate the production of human monoclonal antibodies. Hybridoma cells may be prepared by fusing spleen cells from an immunised animal with a tumour cell. Appropriately secreting hybridoma cells may thereafter be selected.

Particulate antigens produced in accordance with the invention may be useful in the preparation of vaccines, for example immunotherapeutic vaccines, which form a further aspect of the invention. The vaccine may comprise a particulate antigen and a physiologically acceptable non-toxic carrier, such as sterile physiological saline or sterile PBS. Sterility will generally be essential for parenterally administrable vaccines. One or more appropriate adjuvants may also be present. Examples of suitable adjuvants include muramyl peptide compounds such as prototype muramyl dipeptide, aluminium hydroxide and saponin. For induction of a

CTL response, it may be preferred to immunise in the absence of adjuvant.

Fusion protein and particulate antigens produced by this invention are useful as diagnostic reagents. Particulate antigens for diagnostic purposes are particularly advantageous because they can be physically separated by centrifugation or filtration and can be directly dispersed on solid supports such as glass or plastic slides, dip sticks, macro or micro beads, test tubes, wells of microtitre plates and the like. The particulate antigens of this invention may also be dispersed in fibrous materials such as absorbent disk (US-A-4,632,901), strips or chromatography columns as the solid support. The particles and fusion proteins readily adhere to solid supports. The particles may after purification be disrupted into fusion proteins and the fusion proteins may be dispersed on surfaces as indicated above. These reagents are useful for a variety of diagnostic tests. For example, a test sample suspected of having antibody to the particulate antigen and fluorescent, enzyme or radio-labelled antibody is competitively reacted with the particulate antigen or fusion protein on a solid support and the amount of labelled antibody which binds to the particulate antigen on the solid support.

Particulate antigens of this invention are also useful for labelling target cells to test effector cells for cytotoxic T-Lymphocyte activity. Particulate antigens of this invention are also useful for agglutination reactions with antibodies. Those skilled in the diagnostic arts will recognise a wide variety of application of particulate antigens and fusion proteins of this invention for diagnostic purposes.

The *gag* expression cassette described above may also be used as a research tool to investigate for example, retroviral morphogenesis, Tat-TAR and Rev/RRE interactions, and splicing mechanisms, and to develop screens which identify inhibitors of these proteins. Cells may be transiently or stably transfected with the *gag* expression cassette to create cells which have many of the characteristics of HIV-transfected cells but which are safe to manipulate.

Preferred features for each aspect of the invention are as for each of the other

aspects *mutatis mutandis*.

The following examples illustrate the invention, but are not intended to limit the scope in any way. The examples refer to:

FIGURE 1, which shows restriction maps of pGC559, pGC560, pGC561 and pGC562

Example 1. Construction of the *gag* Portion of the Deleted Virus

The 5' portion of the construct contains the 5' untranslated region of the viral genome, the *gag* and *protease* genes, and possesses a number of alterations as follows. The 19 bp deletion which significantly reduces the incorporation of viral RNA into the virion (Lever *et al.*, J. Virol 1989 63 4085-4087) is used to inhibit construct-derived RNA being packaged into the recombinant Gag particles. One *Pst*I and two *Hind*III restriction sites are mutated to facilitate DNA manipulation, and a *Pst*I site, a *Bam*HI site, translational stops and a linker sequence are inserted 3' of the protease gene to allow subcloning of the *gag* DNA fragment and replacement of the 3' portion of the p7 gene with p7 fusions.

The ca. 2Kb *Sac*I-*Msc*I DNA fragment from pOGS1, which encodes the *gag-protease* region of the HIV genome, was subcloned into *Sac*I- and *Hinc*II- cut M13mp18 to provide a template for mutagenesis (pLGC25). Three mutations to remove two *Hind*III and one *Pst*I restriction sites were simultaneously introduced using oligonucleotides:

24-mer 5'-CTTGTCTAAAGCCTCCTTGGTGTC-3',
21-mer 5'- CCTGTGAAGCCTGCTCGGCTC-3' and
33-mer 5'- CTCTATCCCATTCTGCGGCTTCCTCATTGATGG-3'.

A clone containing all three mutations (pLGC26) was identified by restriction endonuclease analysis of RF form DNA. pLGC26 was used as a template for further mutagenesis with a 63-mer:

5'-CAGTCTCAATAGGGCTAATGGGCTGCAGCTAGGACCGGATCCTTAAAA ATTTAAAGTGCAACC-3',

which placed a stop codon, a *Bam*HI site, codons for arginine and serine, a second stop codon and *Pst*I site at the end of the protease gene to produce mutant pLGC27. In addition to the required changes, a distant deletion of a single base was identified which required repairing. pLGC27 was used as a template for mutagenesis with a 26-mer:

5'-GGTGCAATAGGCCCTGCATGCACTGG-3',

which was designed to repair the single base deletion, and a 39-mer:

5'-CCATCTCTCTCCTTCTAGCCGGCGTACTCACCAGTCGCC-3',

which deleted the 19 bases in the 5' untranslated leader sequence involved in packaging RNA into the virion. A clone containing both mutations, pGC554, was identified by DNA sequencing.

Example 2 Construction of the Tat/Rev/RRE Portion of th Deleted Virus

The construction of the 3' portion of the deleted virus involves cloning the RRE element into a polylinker and then subcloning the 5' and 3' portions of the Tat-Rev encoding DNA, 5' and 3' of the RRE element respectively. Thus the natural RNA splicing pattern is maintained but the distance is altered. Mutations designed to optimize the 3' end of the construct include mutation of a gp120 internal ATG codon to suppress potential ORF internal translational initiation, and removal of a *Bam*HI site to aid subcloning.

The 368 bp *Sau*IIa DNA fragment from pOGS1, which encodes the RRE sequence, was cloned into *Bam*HI- cut M13mp19 to generate pGC550. pGC550 was used as a template for mutagenesis with oligonucleotide 30-mer:

5'- CCAATTGTCCCTCAAATCTCCTCCTCCAGG-3',

which was designed to mutate an ATG internal to the gp120 ORF to ensure that gp120 sequences will not be expressed. A correct clone pGC551 was identified by DNA sequencing. The ca. 755 bp *Hind*III-*Xho*I DNA fragment from pOGS1, which encodes the C-terminal portion of the Tat and Rev proteins, was cloned into pGC551. pOGS1 DNA was cut with restriction endonucleases *Hind*III, *Xho*I and *Bg*1II. The ca. 755 bp DNA fragment was gel-purified and the ends made blunt by filling in with Kenow and dNTPs. The blunt fragment was cloned into pGC551 cut with *Bam*HI and similarly blunt ended with Kenow and dNTPs to create pGC552.

pGC552 was used as a template for mutagenesis with oligonucleotide33-mer:

5'-CCAGATAAGTGCTAAGGATACGTTCACTAATCG-3'

which was designed to mutate a *Bam*HI restriction site. The correct clone pGC555 was identified by DNA sequencing. The DNA encoding the 5' ORFs of Tat and Rev

was cut from pOGS1 with *Eco*RI and *Ssp*1 and was made blunt by filling in with Klenow and dNTPs. The blunt fragment was cloned into pGC555 which had been cut with *Xba*I and similarly blunt-ended. A clone in which the insert was in the correct orientation, pGC556, was identified by DNA sequencing. pGC5566 was used as a template for two rounds of oligonucleotide directed mutagenesis. First, oligonucleotides:

23-mer 5'-CAGTTGTTGCAGAGTCGACCTGC-3' and
22-mer 5'-CTGAAGATCCTATTTTCCTATA-3'

were used to mutate the 5' *Eco*RI and *Xba*I and the 3' *Xba*I sites recreated during he construction of pGC556. A clone which contained the desired mutations was then used as a template for mutagenesis with oligonucleotide:

23-mer 5'-CAGTGAATTCGCGGTACCCGGGG-3'

which was designed to mutate the 3, *Sst*I site in the polylinker of the vector. A correct clone, pGC557, was identified by DNA sequencing. pGC557 contains the completed 3' portion of the deleted virus construct.

Example 3 Sequencing of the 5' and 3' Portions of the Deleted Virus Construct

Both the 5' portion of the construct (pGC554) containing the *gag* and *protease* genes and the 3' portion of the construct (pGC557) containing the Tat/Rev and RRE sequences were sequenced. This showed that no sequence alterations had arisen during the construction of the two halves of the deleted virus.

Example 4 Construction of the Deleted Virus from two halves

The 5' portion of the deleted virus containing the *gag* genes was modified and cloned into pUC18. The construction was completed by the addition of the 3' portion of the deleted virus containing the self-regulating *tat*, *rev* and RRE cassette.

Replicative form DNA of pGC554 was digested with restriction endonucleases *Sac*I and *Pst*I. A ca. 1900bp DNA fragment was isolated and cloned into *Hind*III and *Pst*I cut pUC18 DNA. A 10 bp linker (5'-AGCTTGAGCT-3') was used to adapt the two incompatible *Hind*III and *Sac*I sites. A correct clone pGC558 was identified by DNA sequencing.

pGC558 DNA was cut with restriction endonucleases PstI and EcoRI and used as a vector to clone the *tat/rev/RRE* containing *Pst*I-*Eco*RI ca. 1700bp DNA fragment from pGC557. A correct clone, pGC559, was identified by DNA sequencing. pGC559 is the full-length version of the deleted virus and contains DNA encoding p7, p6 and protease and is shown in Figure 1.

Example 5 Construction of Variants of pGC559

Restriction maps of these variants are shown in Figure 1.

pGC560 contains DNA encoding p7 and p6 but most of the protease gene has been deleted. The ca. 450bp *Bg*/II-*Bam*HI DNA fragment from pGC559 was replaced with a ca. 270bp *Bg*/II-*Bam*HI DNA fragment from pAcKGAG.

pGC561 contains the p7-V3 fusion but has p6 and protease deleted. The ca. 540bp *Apa*I-*Bam*HI DNA fragment from pGC559 has been replaced with a 228 bp *Apa*I-*Bam*HI DNA fragment from pOGS574.

pGC562 has a deletion of p6 and protease to leave the p7 coding sequence intact. The ca. 450bp *Bg*/II-*Bam*HI DNA fragment from pGC559 was removed and the vector backbone religated.

Example 6: Expression analysis in mammalian cells

The *gag/rev*/RRE cassette was released from pGC560 as a *HindIII* to *EcoRI* fragment and cloned into the mammalian expression vector pGW1HG (also digested with *HindIII/EcoRI*) to generate pOGS754. The vector pGW1HG (disclosed in co-pending patent application WO-A-9109118) is a generic mammalian cell expression vector consisting of a pUC19 backbone containing an hCMV promoter with associated polylinker for insertion of foreign gene and SV40 polyadenylation signal. The vector also includes an SV40 origin of replication and a *gpt* cassette (containing an SV40 early promoter, a *gpt* gene and SV40 polyadenylation signal). When cloned using the restriction enzyme sites described, the *gag* cassette comes under the control of the hCMV promoter. The *gag,protease/RRE/rev* cassette was released from pGC559 and similarly cloned into pGW1HG as a *HindIII* to *EcoRI* fragment to generate pOGS753.

Purified plasmid DNA was transfected into COS-7 cells for transient expression analysis using the following DEAE-Dextran method. Cells in log growth phase were used to seed 175cm^2 tissue culture flasks with 1.5x10^6 cells each. After a recovery period of 24h the medium (DMEN + 2mM glutamine + 100IU/ml penicillin, 100mg/ml streptomycin + 10% heat-inactivated fetal calf serum) was discarded, and the cells washed three times with PBS B. Following removal of the last PBS B wash, 18ml of freshly prepared transfection mix was added, and the cells incubated at 37°C for 3h in 8% CO_2 atmosphere. The transfection mix consisted of 210µl of TE (10mM Tris-HCl, 1mM EDTA, pH8.0) containing 18µg of plasmid DNA, 18µl chloroquine (100mM in TE), 72µl DEAE-Dextran (100mg/ml in TE) and 17.7ml of growth medium (DMEN + 2mM glutamine + 100U/ml penicillin/ streptomycin + 5% Ultraser G). Reagents were mixed in this order. After the 3h incubation period the transfection mix was discarded, and the cells exposed to 18ml of 10% DMSO (in PBS) for 2min at room temperature. This was then removed and the cells washed twice with PBS B before addition of 50ml growth medium. At 48h post-transfection the cells were harvested for analysis by immunofluorescence and electron microscopy.

For immunofluorescence studies, aliquots of transfected cells were dried on microscope slides then fixed in acetone/methanol (1:1). After washing in PBS, cells were probed with mouse polyclonal anti-Gag anti-serum or an anti-Rev monoclonal antibody (No. 196010, supplied by Applied Biotechnologies), followed by FITC-labelled goat anti-mouse IgG (Sigma, Poole, UK). The results from these experiments show high level expression of both Gag and Rev in pOGS754-transfected cells. As anticipated, Rev protein accumulated in the nucleus. Confirmation that Gag expression results in the formation of particles which bud from the cell membrane was provided by electron microscopy.

Construct pOGS753 contains the *gag,protease/Rev*/RRE cassette. To confirm that the protease is active in this construct, cells were transfected with pOGS753 or pOGS754 as described, and cell lysates together with budded Gag particles (pelleted from the culture supernatants by centrifugation) were examined by Western blot analysis with a pool of human HIV-positive antisera. Bands corresponding to the Gag components generated by protease cleavage were observed.

Example 7 Chloramphenicol acetyl transferas assay

The constructs described in this application have been designed to express Tat to optimise expression from a Tat-sensitive promoter (eg. HIV LTR). COS-7 cells were co-transfected with varying amounts of pOGS753 DNA together with 1μg of pOGS210 DNA (chloramphenicol acetyl transferase (CAT) gene under control of an HIV-1 LTR). Synthesis of Tat from pOGS753 activates the LTR promoter and results in expression of CAT which is detected by a CAT assay. After 48th CAT assays were performed with cell extracts, and the % conversion of chloramphenicol to acetylated forms quantified using a phosphoimager. As a positive control cells were co-transfected with pOGS210 and pOGS213 (chloramphenicol acetyl transferase gene under control of a CMV promoter), while cells transfected with pOGS213 alone acted as a negative control. Table 1 shows the result from this assay and demonstrates expression of Tat.

Table 1

	pOGS753 10ng	pOGS753 50ng	pOGS753 100ng	NO DNA (CONTROL)	pOGS213 10ng
% CONVER-SION	7.2%	29.4%	35.8%	0.3%	53.0%

CLAIMS

1 A DNA construct comprising:

(a) a single promoter;
(b) a *gag*-derived sequence from a retrovirus,
(c) a *rev*-like element;
(d) an RRE-like element; and
(e) donor and acceptor elements;

wherein

(i) elements (a) to (e) are arranged in such a fashion that in the construct the promoter is capable of driving expression of both the *gag*-derived sequence and, by virtue of the donor and acceptor elements, the *rev*-like element;

(ii) the construct does not contain a functional *env* gene.

2 A DNA construct as claimed in claim 1 where the retrovirus is of lentiviral origin.

3 A DNA construct as claimed in claim 2 where the retrovirus is of spumaretroviral origin.

4. A DNA construct as claimed in any of claims 1-2 where the retrovirus is Human Immunodeficiency Virus, Simian Immunodeficiency Virus or Bovine Immunodeficiency-like Virus (BIV).

5 A DNA construct as claimed claims 1 or 3 where the retrovirus is simian foaming virus, bovine syncitial virus, or feline syncitial virus.

6 A DNA construct as claimed in any of claims 1-5 where the *gag* sequence is supplemented by one or more additional codons coding for amino acids.

7 A DNA construct as claimed in any of claims 1-6 where the *gag* sequence comprises part of the retroviral *polymerase* gene.

8 A DNA construct as claimed in claim 7 where the part of the *polymerase* gene comprises only the protease-encoding moiety.

9 A DNA construct as claimed in any of claims 1-8 where the *rev*- like element is derived from a complex retrovirus.

10 A DNA construct as claimed in claim 9 where the *rev*- like element is derived from the same retrovirus as the *gag* element.

11 A DNA construct as claimed in any of claims 1-10 where the RRE-like element is derived from a complex retrovirus.

12 A DNA construct as claimed in claim 11 where the RRE- like element is matched to the *rev*- like element.

13 A DNA construct as claimed in any of claims 1-12 where the selected RNA splicing donor and acceptor sites are positioned in the natural linear sequences but in a spatially compacted configuration.

14 A DNA construct as claimed in any of claims 1-13 comprising a minimal amount of *env* compatible with convenient manipulation of *rev* and RRE.

15 A DNA construct as claimed in claim 14 comprising a deletion of greater than 50% of the *env* gene.

16 A DNA construct as claimed in any of claims 1-15 which includes the Tat-

TAR interaction sequences.

17 A DNA construct as claimed in claim 16 utilising as a promoter the HIV-LTR sequence containing the TAR site.

18 A DNA construct as claimed in any of claims 1-17 comprising a further antigenic sequence in an open reading frame with the *gag* gene.

19 A DNA construct as claimed in any of claims 1-18 where the antigenic sequence is found at the 3' end of the p6, p7, p24 or p17 region of the *gag* gene.

20 A DNA construct as claimed in any of claims 1-19 where the antigenic sequence is inserted within an epitope that is surface exposed when the Gag protein forms particles.

21 A DNA construct as claimed in any of claims 1-20 where the antigenic sequence corresponds to a sequence derived from or associated with an aetiological agent or a tumour.

22 A DNA construct as claimed in claim 21 where the aetiological agent is a microorganism such as a virus, bacterium, fungus or parasite.

23 A DNA construct as claimed in claim 22 where the virus is a retrovirus, such as HIV-1, HIV-2, HTLV-I, HTLV-II, HTLV-III, SIV, BIV, LAV, ELAV, CIAV, murine leukaemia virus, Moloney murine leukaemia virus, and feline leukaemia virus; an orthomyxovirus, such as influenza A or B; a paramyxovirus, such as parainfluenza virus, mumps, measles, RSV and Sendai virus; a papovavirus, such as HPV; an arenavirus, such as LCMV of humans or mice; a hepadnavirus, such as Hepatitis B virus; a herpes virus, such as HSV, VZV, CMV, or EBV.

24 A DNA construct as claimed in claim 22 where the antigenic sequence is derived from a bacterium, such as of the genus *Neisseria, Campylobacter, Bordetella, Listeria, Mycobacteria* or *Leishmania,* or a parasite, such as from the genus *Trypanosoma, Schizosoma, Plasmodium,* especially *P. falciparum,* or from a fungus, such as from the genus *Candida, Aspergillus, Cryptococcus, Histoplasma* or *Blastomyces.*

25 A DNA construct as claimed in claim 22 where the antigenic sequence is a proteinaceous human tumour antigen, such as a melanoma-associated antigen, or an epithelial-tumour associated antigen such as from breast or colon carcinoma.

26 A DNA construct as claimed in claim 22 where the antigenic sequence is an epitope from:

1) HIV (particularly HIV-1) gp120,
2) VZV gpI, gpII and gpIII
3) Influenza virus nucleoprotein and haemagglutinin,
4) LCMV nucleoprotein,
5) HPV L1, L2 E4, E5, E6 and E7 proteins,
6) p97 melanoma associated antigen,
7) GA 733-2 epithelial tumour-associated antigen,
8) MUC-1 epithelial tumour-associated antigen,
9) Melanoma MZ2-E antigens,
10) Malaria CSP or RESA antigens,
11) Mycobacterium p6

27 A DNA construct as claimed in claim 26 where the antigenic sequence is third variable domain of a lentivirus.

28 A DNA construct as claimed in claim 27 where the lentivirus is HIV-1.

29 A process for the preparation of a construct as claimed in any one of claims 1-28, the process comprising ligating, in one or a plurality of steps, two or more DNA segments between them comprising in any combination:

(a) a single promoter;
(b) a *gag*-derived sequence from a complex retrovirus,
(c) a *rev*-like element;
(d) an RRE-like element
(e) donor and acceptor elements;

the segments being ligated in such a fashion that:

(i) in the construct the promoter is capable of driving expression of both the *gag*-derived sequence and, by virtue of the donor and acceptor elements, the *rev*-like element; and
(ii) the construct does not contain a functional *env* gene.

30 A process as claimed in claim 29 where the *rev*-like element and the RRE-like element are first ligated together before being joined to the promoter and *gag*-derived sequence.

31 A DNA construct consisting substantially only of:

(a) a *rev*-like element;
(b) an RRE-like element; and
(c) donor and acceptor elements.

32 A vector including a nucleic acid as claimed in any of claims 1-29.

33 A vector as claimed in claim 32 where the vector is derived from a plasmid.

34 A vector as claimed in claim 32 where the vector is derived from a live non-oncogenic virus.

35 Mammalian host cells transformed with vectors as claimed in any of claims 32-34.

36 Mammalian host cells as claimed in claim 35 where the host cells are COS cells, Chinese Hamster Ovary (CHO) cells, mouse myeloma cell lines such as P3X63-Ag8.653, HeLa cells, BHK cells, VERO cells, melanoma cells such as the Bowes cell line, mouse L cells, human hepatoma cell lines such as HepG2, mouse fibroblasts or mouse NIH 3T3 cells.

37 Mammalian host cells as claimed in any of claims 35-36 which have also been transformed to co-express targeting proteins expressed as membrane bound proteins which are picked up when the particle buds.

38 A host cell as claimed in claim 37 where the membrane-bound protein confers on the membrane protein i) an immunogenic ability (or improved or altered immunogenic ability) ; ii) a site- or cell-specific targeting ability; iii) fusogenic properties; iv) binding or packaging activity; v) cytotoxicity; vi) pharmaceutical activity; and/or vii) nucleic acid binding activity.

39 A host cells as claimed in any of claims 37-38 where the membrane bound protein is CD4, CD8, ICAM-1, ICAM-2, ICAM-1ß, or VCAM.

40 A host cells as claimed in any of claims 37-39 where the targeting proteins are expressed from genes engineered onto *gag* expression vector variants and expressed from another splice acceptor-donor combination within the *gag* expresion construct.

41 A process for the preparation of proteins encoded at least in part by a *gag*-derived gene from a complex retrovirus (or sequence substantially homologous thereto), the process comprising expressing a DNA construct in accordance with claims 1-28

42 Proteins prepared by the process claimed in claim 41.

43 Particles comprising a plurality of proteins as claimed in claim 42.

44. Antibodies raised against proteins or particles as claimed in claims 42 or 43.

45. The use of proteins or particles as claimed in claims 42 or 43 in the preparation of an immunotherapeutic or prophylactic vaccine.

46. The use of proteins or particles as claimed in claims 42 or 43 as a diagnostic agent.

47. A pharmaceutical or veterinary composition comprising proteins or particles as claimed in claims 42 or 43 together with a pharmaceutically and/or veterinarily acceptable carrier.

48 A DNA construct as shown in Figure 1.

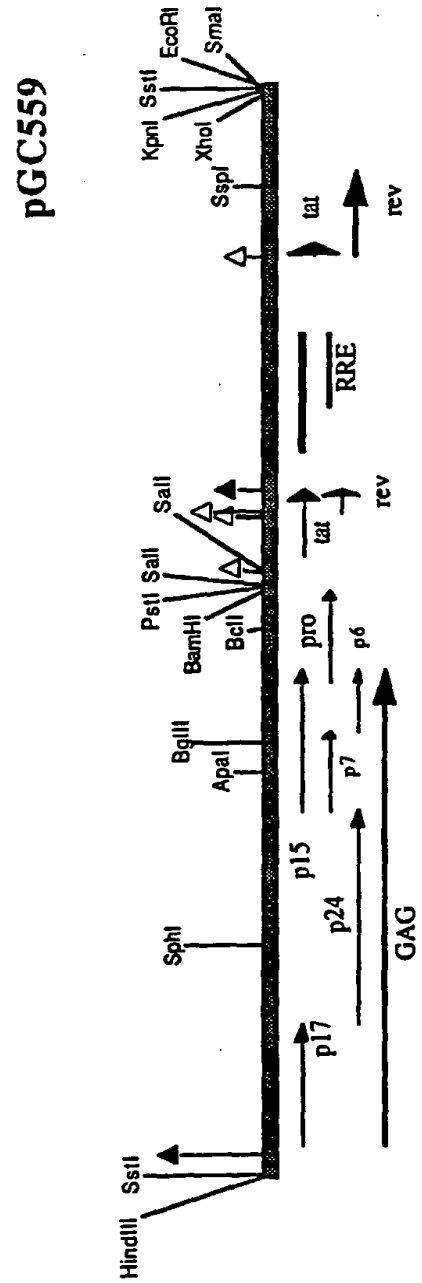
pGC559
EcoRI
SmaI
SstI
KpnI
XhoI
SspI
tat
rev
RRE
SalI
PstI
BamHI
BclI
pro
p6
BglII
ApaI
p7
p15
p24
GAG
SphI
p17
SstII
HindIII

Fig 1

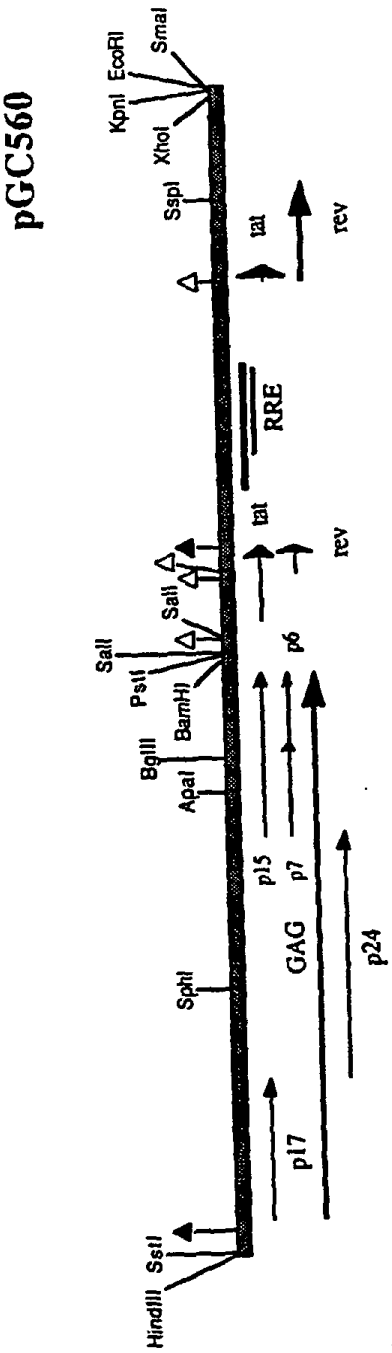
pGC560
EcoRI
SmaI
KpnI
XhoI
SspI
tat
rev
RRE
tat
rev
SalI
SalI
PstI
BamHI
BglII
ApaI
p6
p15
p7
GAG
p24
SphI
p17
SstI
HindIII

Fig 2

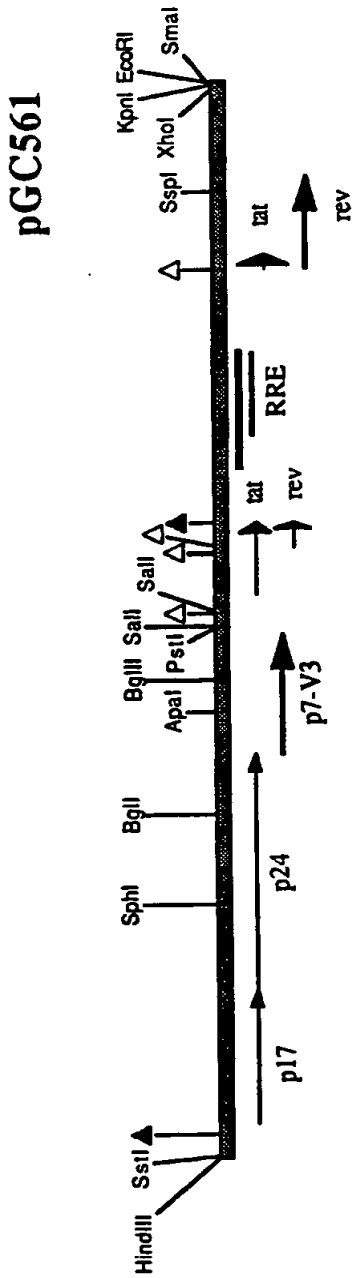
pGC561
KpnI
EcoRI
SmaI
XhoI
SspI
tat
rev
RRE
tat
rev
SalI
SalI
BglII
PstI
ApaI
p7-V3
BglI
SphI
p24
p17
SstI
HindIII

Fig 3

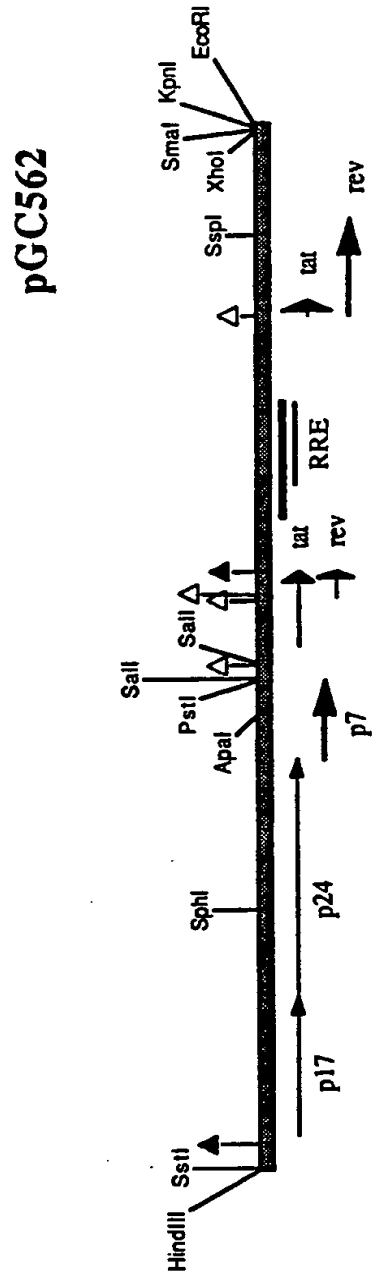
pGC562
EcoRI
KpnI
SmaI
XhoI
SspI
tat
rev
RRE
tat
rev
SalI
SalI
SalI
PstI
ApaI
p7
SphI
p24
p17
SstI
HindIII

Fig 4